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SEARCH REQUEST FORM

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Date: _____ Phone: _____ Art Unit: _____

Search Topic:
Please write a detailed statement of search topic. Describe specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples or relevent citations, authors, keywords, etc., if known. For sequences, please attach a copy of the sequence. You may include a copy of the broadest and/or most relevent claim(s).

Shears, Beverly 114840

From: Devi, Sarvamangala
Sent: Friday, February 20, 2004 11:16 AM
To: Shears, Beverly
Subject: 09/870,122

Beverly:

Would you please perform inventor name searches for the following two inventors in 09/870,122? Please include all conference/meeting databases.

CLEARY, PAUL PATRICK; and STAFSLIEN, DEBORAH K.

Thanks.

S. DEVI, Ph.D.
AU 1645

Scientific & Technical Information Center
USPTO

STIC Search Report
Biotech-Chem Library

STIC Database Tracking Number: 114840

To: Sarvamangala Devi
Location: REM 3C18
Art Unit: 1645
Friday, February 20, 2004

Case Serial Number: 09/870122

From: Beverly Shears
Location: Remsen Bldg.
RM 1A54
Phone: 571-272-2528

beverly.shears@uspto.gov

STAFF USE ONLY

Date completed: 02-20-04
Searcher: Rebecca 2528
Terminal time: _____
Elapsed time: _____
CPU time: _____
Total time: _____
Number of Searches: _____
Number of Databases: 2

Search Site
_____ STIC
_____ CM-1
_____ Pre-S

Type of Search
_____ N.A. Sequence
_____ A.A. Sequence
_____ Structure
_____ Bibliographic

Vendors
_____ IG
✓ STN
✓ Dialog
_____ APS
_____ Geninfo
_____ SDC
_____ DARC/Questel
_____ Other

PTO-1590 (9-90)

STIC Search Report
Biotech-Chem Library

STIC Database Tracking Number: 104999

TO: Sarvamangala Devi **Location: CM1/7E15&7E12** **Art Unit: 1645** **Thursday, October 02, 2003** **Case Serial Number: 09/870122**	**From: Edward Hart** **Location: Biotech-Chem Library** **CM1-6B02** **Phone: 305-9203** **edward.hart@uspto.gov**

Search Notes

Examiner Devi,

Here are the results of the search you requested.

Please feel free to contact me if you have any questions.

Edward Hart

104999

STIC-Biotech/ChemLib

From: Devi, Sarvamangala
Sent: Wednesday, October 01, 2003 2:13 PM
To: STIC-Biotech/ChemLib
Subject: 09/870,122

CRF-C

STIC-Biotech/ChemLib:

Please perform a sequence and an interference search for SEQ ID NO: 1, 2, 3 and 23 and an oligopeptide comprising at least 7 amino acid-long fragment thereof, in application SN 09/870,122.

Thanks.

S. DEVI, Ph.D.
AU 1645

Searcher:_____
Phone:_____
Location:_____
Date Picked Up: 10/8/03
Date Completed:_____
Searcher Prep/Review:_____
Clerical:_____
Online time:_____

TYPE OF SEARCH:
NA Sequences: 8
AA Sequences:_____
Structures:_____
Bibliographic:_____
Litigation:_____
Full text:_____
Patent Family:_____
Other:_____

VENDOR/COST (where applic.)
STN:_____
DIALOG:_____
Questel/Orbit:_____
DRLink:_____
Lexis/Nexis:_____
Sequence Sys.: PD
WWW/Internet:_____
Other (specify):_____

Devij S.
09/870122

20feb04 12:46:00 User219783 Session D1993.2

SYSTEM:OS - DIALOG OneSearch
File 65:Inside Conferences 1993-2004/Feb W3
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File 440:Current Contents Search(R) 1990-2004/Feb 20
(c) 2004 Inst for Sci Info
*File 440: New prices as of 1/1/2004 per Information Provider request. See HELP RATES 440.
File 348:EUROPEAN PATENTS 1978-2004/Feb W03
(c) 2004 European Patent Office
File 357:Derwent Biotech Res. 1982-2004/Feb W4
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*File 357: New prices as of 1-1-04 per information provider. See HELP RATES357
File 113:European R&D Database 1997
(c)1997 Reed-Elsevier(UK)Ltd All rts reserv
*File 113: This file is closed (no updates)

Set Items Description
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Set Items Description — Author(s)
S1 682 AU=(CLEARY, P? OR CLEARY P?)
S2 9 AU=(STAFSLIEN D? OR STAFSLIEN, D?)
S3 9 S1 AND S2
S4 42 (S1 OR S2) AND ((STREPTOCOCC? OR GAS)(3N)PEPTIDASE OR SCPA)
S5 42 S2 OR S4
S7 15 S5 AND (IMMUNIS? OR IMMUNIZ? OR VACCIN?)
S8 20 S3 OR S7
S9 11 RD (unique items)
>>>No matching display code(s) found in file(s): 65, 113

9/3,AB/1 (Item 1 from file: 65)
DIALOG(R)File 65:Inside Conferences
(c) 2004 BLDSC all rts. reserv. All rts. reserv.

04854436 INSIDE CONFERENCE ITEM ID: CN050631320
The group A **streptococcal** C5a **peptidase**, a **vaccine** to obstruct access to tonsils, a reservoir for recurrent infection
Cleary, P. P.; Costalonga, M.; Park, H.-S.
CONFERENCE: Microbial pathogenesis & host response-Meeting
ABSTRACTS OF PAPERS PRESENTED AT THE COLD SPRING HARBOR MEETING ON MICROBIAL PATHOGENESIS AND HOST RESPONSE, 2003 P: 210
Cold Spring Harbor Laboratory, 2003
LANGUAGE: English DOCUMENT TYPE: Conference Abstracts
CONFERENCE SPONSOR: Cold Spring Harbor Laboratory
CONFERENCE LOCATION: Cold Spring Harbor, NY 2001; Sep (200109) (200109)

9/3,AB/2 (Item 1 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

16928425 Document Delivery Available: 000185215600003 References: 35
TITLE: Immune response to group A **streptococcal** C5a **peptidase**

Searcher : Shears 571-272-2528

in children: Implications for **vaccine** development
AUTHOR(S): Shet A (REPRINT); Kaplan EL; Johnson DR; **Cleary PP**
AUTHOR(S) E-MAIL: shetx002@umn.edu
CORPORATE SOURCE: Univ Minnesota, World Hlth Org Collaborating Ctr Reference & Res, 420 Delaware St SE/Minneapolis//MN/55455 (REPRINT); Univ Minnesota, World Hlth Org Collaborating Ctr Reference & Res, /Minneapolis//MN/55455; Univ Minnesota, Dept Microbiol, /Minneapolis//MN/55455
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF INFECTIOUS DISEASES, 2003, V188, N6 (SEP 15), P 809-817
GENUINE ARTICLE#: 719QN
PUBLISHER: UNIV CHICAGO PRESS, 1427 E 60TH ST, CHICAGO, IL 60637-2954 USA
ISSN: 0022-1899
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The group A **streptococcal** C5a **peptidase** (**SCPA**) is a major surface virulence protein that facilitates the establishment of local infection by group A streptococci (GAS). We measured the human immune response to **SCPA**, using a standardized indirect enzyme-linked immunosorbent assay. Paired acute and convalescent serum samples from children with GAS-associated pharyngitis were assayed, and a strong immune response to **SCPA** was demonstrated that was independent of the infecting M type and the age of the patient. Western blot analysis of bacterial extracts revealed that all tested M types expressed **SCPA**. The immune response to **SCPA** correlated with the anti-streptolysin O and anti-DNase B responses. These data confirm the immunogenicity of **SCPA** in humans. Previous knowledge of SPCA's role in virulence, its highly conserved nature, and the results of mouse protection studies make **SCPA** an ideal **vaccine** candidate for the prevention of GAS disease.

9/3,AB/3 (Item 2 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

14916425 Document Delivery Available: 000178675100058 References: 38
TITLE: **Immunization** with C5a peptidase or peptidase-type III polysaccharide conjugate **vaccines** enhances clearance of group B streptococci from lungs of infected mice
AUTHOR(S): Cheng Q; Debol S; Lam H; Eby R; Edwards L; Matsuka Y; Olmsted SB ; **Cleary PP (REPRINT)**
AUTHOR(S) E-MAIL: cleary@lenti.med.umn.edu
CORPORATE SOURCE: Univ Minnesota, Dept Microbiol, MMC 196,420 Delaware St SE/Minneapolis//MN/55455 (REPRINT); Univ Minnesota, Dept Microbiol, /Minneapolis//MN/55455; Wyeth Lederle Vaccines, /Rochester//NY/14586
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2002, V70, N11 (NOV), P6409-6415
GENUINE ARTICLE#: 605JQ
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Group B streptococci (GBS) are among the most common causes of

life-threatening neonatal infections. **Vaccine** development since the late 1970s has focused on the capsular polysaccharides, but a safe, effective product is still not available. Our quest for a **vaccine** turned to the **streptococcal** C5a **peptidase** (SCPB). This surface protein is antigenically conserved across most if not all serotypes. A murine model was used to assess the impact of SCPB on clearance of GBS from the lungs of intranasally infected animals. Mutational inactivation of SCPB resulted in more-rapid clearance of streptococci from the lung. **Immunization** with recombinant SCPB alone or SCPB conjugated to type III capsular polysaccharide produced serotype-independent protection, which was evidenced by more-rapid clearance of the serotype VI strain from the lungs. **Immunization** of mice with tetanus toxoid-type III polysaccharide conjugate did not produce protection, confirming that protection induced by SCPB conjugates was independent of type III polysaccharide antigen. Histological evaluation of lungs from infected mice revealed that pathology in animals **immunized** with SCPB or SCPB conjugates was significantly less than that in animals **immunized** with a tetanus toxoid-polysaccharide conjugate. These experiments suggest that inclusion of C5a peptidase in a **vaccine** will both add another level to and broaden the spectrum of the protection of a polysaccharide **vaccine**.

9/3,AB/4 (Item 3 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

14000216 Document Delivery Available: 000175761400077 References: 1
TITLE: The group B streptococcal C5a peptidase is both a specific protease and an invasin (vol 70, pg 2408, 2002)
AUTHOR(S): Cheng Q (REPRINT); **Stafslien D**; Purushothaman SS; **Cleary P**
CORPORATE SOURCE: Univ Minnesota, Dept Microbiol, /Minneapolis//MN/55455 (REPRINT); Univ Minnesota, Dept Microbiol, /Minneapolis//MN/55455
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2002, V70, N6 (JUN), P3309-3309
GENUINE ARTICLE#: 554XA
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: CORRECTION

9/3,AB/5 (Item 4 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

12548620 References: 40
TITLE: Antibody against surface-bound C5a peptidase is opsonic and initiates macrophage killing of group B streptococci
AUTHOR(S): Cheng Q; Carlson B; Pillai S; Eby R; Edwards L; Olmsted SB; **Cleary P (REPRINT)**
AUTHOR(S) E-MAIL: cleary@lenti.med.umn.edu
CORPORATE SOURCE: Univ Minnesota, Dept Microbiol, Box 196 UMHC/Minneapolis//MN/55455 (REPRINT); Univ Minnesota, Dept Microbiol, /Minneapolis//MN/55455; Wyeth Lederle Vaccine, /Rochester//NY/

Searcher : Shears 571-272-2528

PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 2001, V69, N4 (APR), P2302-2308
GENUINE ARTICLE#: 413MT
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: The capsular polysaccharides of group B streptococci (GBS) are a primary focus of **vaccine** development, Immunogenicity and long-lasting protection are best achieved by conjugating polysaccharides to a T-cell-dependent protein antigen. **Streptococcal** C5a **peptidase** (SCPB) is a conserved surface protein that is expressed by all streptococcal serotypes tested to date, and it is a possible carrier protein that could itself induce a protective immune response, Clearance of GBS from lungs, mucosal surfaces, or blood probably depends on the opsonophagocytic response of tissue-specific macrophages and polymorphonuclear leukocytes (PMNs), In this study, we examined the potential of antibody directed against SCPB from a serotype II. strain to enhance the capacity of mouse bone marrow macrophages (from primary cultures) and human PMNs in whole blood to kill GBS in vitro, Our experiments demonstrated that Streptococcus serotypes Ia, Ib, II, III, and V, preopsonized with anti-SCPB antibody, were killed more rapidly by cultured macrophages and PMNs in whole blood than were nonopsonized GBS. The increased rate of killing was accompanied by an increased macro phage oxidative burst. Furthermore, opsonization was serotype transparent. **Immunization** with SCPB conjugated to capsular polysaccharide type III produced polysaccharide-specific antibodies. It is interesting that this antiserum promoted serotype-independent killing of streptococci. These data support the use of SCPB in a GBS polysaccharide conjugate **vaccine**. SCPB not only enhanced the immunogenicity of polysaccharide components of the **vaccine**, but it might also induce additional serotype-independent protective antibodies.

9/3,AB/6 (Item 5 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

11629135 References: 31
TITLE: Characterization of the streptococcal C5a peptidase using a C5a-green fluorescent protein fusion protein substrate
AUTHOR(S): **Stafslien DK; Cleary PP (REPRINT)**
AUTHOR(S) E-MAIL: Cleary@lenti.med.umn.edu
CORPORATE SOURCE: Univ Minnesota, Dept Microbiol, Box 196 FUMC,420 Delaware St SE/Minneapolis//MN/55455 (REPRINT); Univ Minnesota, Dept Microbiol, /Minneapolis//MN/55455
PUBLICATION TYPE: JOURNAL
PUBLICATION: JOURNAL OF BACTERIOLOGY, 2000, V182, N11 (JUN), P3254-3258
GENUINE ARTICLE#: 313EU
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA
ISSN: 0021-9193
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: A glutathione-S-transferase (GST)-C5a-green fluorescent protein

(GFP) fusion protein was designed for use as a substrate for the streptococcal C5a peptidase (SCPA), The substrate was immobilized on a glutathione-Sepharose affinity matrix and used to measure wild-type SCPA activity in the range of 0.8 to 800 nM, The results of the assay demonstrated that SCPA is highly heat stable and has optimal activity on the synthetic substrate at or above pH 8.0. SCPA activity was unaffected by 0.1 to 10 mM Ca2+, Mg2+, and Mn2+ but was inhibited by the same concentrations of Zn2+, The assay shows high sensitivity to ionic strength; NaCl inhibits SCPA cleavage of GST-C5a-GFP in a dose-dependent manner. Based on previously published computer homology modeling, four substitutions were introduced into the putative active site of SCPA: Asp(130)-Ala, His(193)-Ala, Asn(295)-Ala, and Ser(512)-Ala. All four mutant proteins had over 1,000-fold less proteolytic activity on C5a in vitro, as determined both by the GFP assay described here and by a polymorphonuclear cell adherence assay. In addition, recombinant SCPA1 and SCPA49, from two distinct lineages of Streptococcus pyogenes (group A streptococci), and recombinant SCPB, from Streptococcus agalactiae (group B streptococci), were compared in the GFP assay. The three enzymes had similar activities, all cleaving approximately 6 mol of C5a mmol of SCP-1 liter(-1) min(-1).

9/3,AB/7 (Item 6 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

09953252 References: 31
TITLE: Impact of M49, mrp, enn, and C5a peptidase proteins on colonization of the mouse oral mucose by Streptococcus pyogenes
AUTHOR(S): Ji YD; Schnitzler N; DeMaster E; **Cleary P (REPRINT)**
CORPORATE SOURCE: UNIV MINNESOTA,DEPT MICROBIOL, BOX 196 FUMC/MINNEAPOLIS//MN/55455 (REPRINT); UNIV MINNESOTA,DEPT MICROBIOL/MINNEAPOLIS//MN/55455; UNIV HOSP AACHEN,NATL REFERENCE LAB STREPTOCOCCI/AACHEN//GERMANY/; UNIV HOSP AACHEN,INST MED MICROBIOL/AACHEN//GERMANY/
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1998, V66, N11 (NOV), P5399-5405
GENUINE ARTICLE#: 132HT
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Resistance to phagocytosis is a hallmark of virulent Streptococcus pyogenes (group A **streptococcus**), Surface bound C5a **peptidase** reduces recruitment of phagocytes to the site of infection, and hyaluronic acid capsules and/or the M protein limit the uptake of streptococci, In this study the relative impact of M and M-like proteins and the C5a peptidase on the virulence of a serotype M49 strain was assessed. The capacities of isogenic strains with an insertion mutation in emm49; with a deletion mutation in scpA49 (C5a peptidase gene); and,vith a deletion that removes all three M-like genes, mrp49, emm49, and enn49, to colonize mice and resist phagocytosis were compared. Experiments confirmed results obtained in an earlier study, which showed that the M49 protein was not required for in vitro resistance to phagocytosis, and also showed that the M protein was not required for colonization of mice. Failure to produce all three M-like proteins, M49, Mrp, and Enn49, significantly reduced the

ability of these streptococci to resist phagocytosis in vitro but did not significantly alter the persistence of streptococci on the oral mucosa, In vitro experiments indicate that M+ streptococci are phagocytized by polymorphonuclear leukocytes that have been activated with phorbol-12-myristate 13-acetate or recombinant human C5a, This observation may explain the finding that expression of M49 protein is not essential for short-term colonization of the mouse oral mucosa.

9/3,AB/8 (Item 7 from file: 440)
DIALOG(R)File 440:Current Contents Search(R)
(c) 2004 Inst for Sci Info. All rts. reserv.

08492686 References: 26
TITLE: Intranasal **immunization** with C5a peptidase prevents nasopharyngeal colonization of mice by the group A Streptococcus
AUTHOR(S): Ji YD; Carlson B; Kondagunta A; **Cleary PP (REPRINT)**
CORPORATE SOURCE: UNIV MINNESOTA,DEPT MICROBIOL, BOX 196 UMHC/MINNEAPOLIS//MN/55455 (REPRINT); UNIV MINNESOTA,DEPT MICROBIOL/MINNEAPOLIS//MN/55455
PUBLICATION TYPE: JOURNAL
PUBLICATION: INFECTION AND IMMUNITY, 1997, V65, N6 (JUN), P2080-2087
GENUINE ARTICLE#: XB562
PUBLISHER: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
ISSN: 0019-9567
LANGUAGE: English DOCUMENT TYPE: ARTICLE

ABSTRACT: Early inflammatory events are initiated by phased production of C5a and interleukin-8 in tissue. Most serotypes of group A streptococci express a surface-bound peptidase (**SCPA**) which specifically cleaves mouse and human C5a chemotaxins. This study investigates the impact of **SCPA** on colonization of the nasopharyngeal mucosa of mice and evaluates its potential to induce protective immunity. Two strains, serotypes M6 and M49, which contain insertion and deletion mutations in the **SCPA** gene (**scpA**) and represent the two major subdivisions of group A streptococci, were characterized and compared in a mouse intranasal infection model. In this model, **SCPA** mutants were more rapidly cleared from the nasopharynges of inoculated mice compared with wild-type strains. A 2,908-bp fragment of scpA49 gene, obtained by PCR, was ligated to the expression vector pGEX-4T-1 and expressed in Escherichia coli. The affinity-purified Delta SCPA49 protein proved to be highly immunogenic in mice and rabbits. Although the purified Delta SCPA49 immunogen lacked enzymatic activity, it induced high titers of rabbit antibodies which were able to neutralize peptidase activity associated with M1, M6, M12, and M49 streptococci in vitro. This result confirmed that antipeptidase antibodies lack serotype specificity. Intranasal **immunization** of mice with the deleted form of the SCPA49 protein stimulated significant levels of specific salivary secretory immunoglobulin A (IgA) and serum IgG antibodies and reduced the potential of wild-type M1, M2, M6, M11, and M49 streptococci to colonize. These experiments suggest a new approach to **vaccine** development for prevention of streptococcal pharyngitis.

9/3,AB/9 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

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01178735
STREPTOCOCCAL C5a **PEPTIDASE VACCINE**
STREPTOKOKKEN-C5A-PEPTIDASE-IMPFSTOFF
VACCIN ANTI-**STREPTOCOCCIQUE** A BASE DE **PEPTIDASE** C5a
PATENT ASSIGNEE:
REGENTS OF THE UNIVERSITY OF MINNESOTA, (267575), 450 McNamara Alumni Center, 200 Oak Street SE, Minneapolis, Minnesota 55455-2070, (US), (Applicant designated States: all)
INVENTOR:
CLEARY, Paul, Patrick, 288 Jansa Drive, Shoreview, MN 55112, (US)
STAFSLIEN, Deborah, K., Apartment 301 5680 East River Road, Fridley, MN 55432, (US
LEGAL REPRESENTATIVE:
Gardner, Rebecca (90041), Frank B. Dehn & Co. 179 Queen Victoria Street, London EC4V 4EL, (GB)
PATENT (CC, No, Kind, Date): EP 1137785 A1 011004 (Basic)
WO 200034487 000615
APPLICATION (CC, No, Date): EP 99966013 991203; WO 99US28826 991203
PRIORITY (CC, No, Date): US 206898 981207
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
EXTENDED DESIGNATED STATES: AL; LT; LV; MK; RO; SI
INTERNATIONAL PATENT CLASS: C12N-015/57; C12N-009/52; A61K-039/09
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English

9/3,AB/10 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
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0256693 DBR Accession No.: 2000-11183 PATENT
Vaccine for streptococcal infection comprises immunogenic amount of variant **streptococcal** C5a-**peptidase** - production of **vaccine** useful for treating disease
AUTHOR: **Cleary P P; Stafslien D K**
CORPORATE SOURCE: Minneapolis, MN, USA.
PATENT ASSIGNEE: Univ.Minnesota 2000
PATENT NUMBER: WO 200034487 PATENT DATE: 20000615 WPI ACCESSION NO.: 2000-423430 (2036)
PRIORITY APPLIC. NO.: US 206898 APPLIC. DATE: 19981207
NATIONAL APPLIC. NO.: WO 99US28826 APPLIC. DATE: 19991203
LANGUAGE: English
ABSTRACT: A new **vaccine** (I) is claimed. (I) comprises a **Streptococcal** C5 a-**peptidase** (SCP1S12A), which is a variant of wild-type SCP, in an amount to **immunize** a susceptible mammal against beta-hemolytic Streptococcus group A, B, C or G. (I) further comprises effective amount of immunological adjuvant and variant SCP1S12A linked to a peptide or polysaccharide. Also claimed are: an isolated and purified peptide containing an enzymatically in active SCP; and an isolated and purified polynucleotide containing a nucleotide sequence encoding an enzymatically in active SCP. (I) is useful for protecting a susceptible mammal, e.g. human, cattle or dog,

against beta-hemolytic Streptococcus, e.g. group A, B, C or G Streptococcus. The application of SCP for **vaccination** reduces the incidence of strep throat and impetigo and also eliminate sequelae such as rheumatic fever, acute glomerulonephritis, sepsis toxic shock and necrotizing fasciitis. (94pp)

9/3,AB/11 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.
(c) 2004 Thomson Derwent & ISI. All rts. reserv.

0230610 DBR Accession Number: 99-00711
Site-directed mutagenesis of the streptococcal C5a peptidase - enzyme production via vector plasmid pGEX-4T-1-mediated scpA gene transfer and expression in Escherichia coli and characterization of activity (conference abstract)
AUTHOR: **Stafslien D K; Cleary P P**
CORPORATE AFFILIATE: Univ.Minnesota
CORPORATE SOURCE: University of Minnesota, Minneapolis, MN, USA.
JOURNAL: Abstr.Gen.Meet.Am.Soc.Microbiol. (98 Meet., 59) 1998
ISSN: 0067-2777 CODEN: 0005P
CONFERENCE PROCEEDINGS: 98th General Meeting of the American Society for Microbiology, Atlanta, GA, USA, 17-21 May, 1998.
LANGUAGE: English
ABSTRACT: The streptococcal C5a peptidase (SCP) is a protein expressed on the surface of group-A streptococci that specifically inactivates C5a, a chemoattractant for neutrophils. It is hypothesized that SCP is a member of the family of subtilisin-like serine proteases based on primary protein sequence analysis. The aim of this study was to verify previously reported computer predictions of the location of the active site amino acids. The scpA gene from serotype M1 strain 90-226 and serotype M49 strain CS101 was amplified using polymerase chain reaction and cloned into the high expression vector plasmid pGEX-4T-1. This fragment coded for the entire mature protein without the membrane anchor domain. The recombinant enzyme was found to have enzymatic activity similar to that of SCP recovered from cell wall extracts of Streptococcus pyogenes. A mutation was introduced into the acpA49 gene, via the megaprimer method of site-directed mutagenesis, which changed the presumed active site serine of the protease to an alanine. The mutation did not effect the protein ability to bind to polyclonal antibodies. The effect of further mutations on enzymatic activity in under investigation. (0 ref)
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20feb04 12:51:25 User219783 Session D1993.3

Devi, S.
09187012 2

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, PHIC, PHIN, TOXCENTER, PASCAL, FEDRIP, DISSABS' ENTERED AT 12:30:09 ON 20 FEB 2004)

L2 1739 SEA ABB=ON PLU=ON "CLEARY P"?/AU — Author(s)
L3 24 SEA ABB=ON PLU=ON "STAFSLIEN D"?/AU
L4 22 SEA ABB=ON PLU=ON L2 AND L3
L5 156 SEA ABB=ON PLU=ON (L2 OR L3) AND ((STREPTOCOCC? OR GAS)(3A) PEPTIDASE OR SCPA(S) STREPTOCOCC?)
L6 31 SEA ABB=ON PLU=ON L5 AND (IMMUNIS? OR IMMUNIZ? OR VACCIN?)
L7 47 SEA ABB=ON PLU=ON L4 OR L6
L8 16 DUP REM L7 (31 DUPLICATES REMOVED)

L8 ANSWER 1 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2003:839352 HCAPLUS
DOCUMENT NUMBER: 140:26685
TITLE: Immune response to group A **streptococcal** C5a **peptidase** in children: implications for **vaccine** development
AUTHOR(S): Shet, Anita; Kaplan, Edward L.; Johnson, Dwight R.; **Cleary, P. Patrick**
CORPORATE SOURCE: Department of Pediatrics, World Health Organization Collaborating Center for Reference and Research on Streptococci, University of Minnesota Medical School, Minneapolis, 55455, USA
SOURCE: Journal of Infectious Diseases (2003), 188(6), 809-817
CODEN: JIDIAQ; ISSN: 0022-1899
PUBLISHER: University of Chicago Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The group A **streptococcal** C5a **peptidase** (**SCPA**) is a major surface virulence protein that facilitates the establishment of local infection by group A **streptococci** (GAS). We measured the human immune response to SCPA, using a standardized indirect ELISA. Paired acute and convalescent serum samples from children with GAS-associated pharyngitis were assayed, and a strong immune response to SCPA was demonstrated that was independent of the infecting M type and the age of the patient. Western blot anal. of bacterial exts. revealed that all tested M types expressed SCPA. The immune response to SCPA correlated with the anti-streptolysin O and anti-DNase B responses. These data confirm the immunogenicity of SCPA in humans. Previous knowledge of SPCA's role in virulence, its highly conserved nature, and the results of mouse protection studies make SCPA an ideal **vaccine** candidate for the prevention of GAS disease.
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2002:180974 HCAPLUS
DOCUMENT NUMBER: 136:246372
TITLE: **Vaccines** comprising **Streptococcal** C5a **peptidase** or

mutants for preventing β-hemolytic Streptococcus colonization or infection

INVENTOR(S): **Cleary, Paul Patrick; Stafslien, Deborah K.**

PATENT ASSIGNEE(S): Régents of the University of Minnesota, USA

SOURCE: U.S., 46 pp., Cont.-in-part of U.S. 5,846,547.
CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6355255	B1	20020312	US 1998-206898	19981207
US 5846547	A	19981208	US 1996-589756	19960122
CA 2243755	AA	19970724	CA 1997-2243755	19970121
US 6270775	B1	20010807	US 1998-206800	19981207
WO 2000034487	A1	20000615	WO 1999-US28826	19991203
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
BR 9915988	A	20010904	BR 1999-15988	19991203
EP 1137785	A1	20011004	EP 1999-966013	19991203
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002531584	T2	20020924	JP 2000-586920	19991203

PRIORITY APPLN. INFO.:
US 1996-589756 A2 19960122
US 1998-206898 A2 19981207
WO 1999-US28826 W 19991203

AB Novel **vaccines** for use against β-hemolytic Streptococcus colonization or infection are disclosed. The **vaccines** contain an immunogenic amount of a variant of **streptococcal** C5a **peptidase** (SCP). Also disclosed is a method of protecting a susceptible mammal against β-hemolytic Streptococcus colonization or infection by administering such a **vaccine**. Enzymically inactive SCP, and polynucleotides encoding these SCP proteins are further disclosed.

REFERENCE COUNT: 73 THERE ARE 73 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 2002:815759 HCAPLUS

DOCUMENT NUMBER: 137:336455

TITLE: **Immunization** with C5a peptidase or peptidase-type III polysaccharide conjugate **vaccines** enhances clearance of group B streptococci from lungs of infected mice

Searcher : Shears 571-272-2528

AUTHOR(S): Cheng, Qi; Debol, Steven; Lam, Hong; Eby, Ron; Edwards, Lorri; Matsuka, Yury; Olmsted, Stephen B.; **Cleary, P. Patrick**
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Infection and Immunity (2002), 70(11), 6409-6415
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Group B streptococci (GBS) are among the most common causes of life-threatening neonatal infections. **Vaccine** development since the late 1970s has focused on the capsular polysaccharides, but a safe, effective product is still not available. The authors' quest for a **vaccine** turned to the **streptococcal** C5a **peptidase** (SCPB). This surface protein is antigenically conserved across most if not all serotypes. A murine model was used to assess the impact of SCPB on clearance of GBS from the lungs of intranasally infected animals. Mutational inactivation of SCPB resulted in more-rapid clearance of streptococci from the lung. **Immunization** with recombinant SCPB alone or SCPB conjugated to type III capsular polysaccharide produced serotype-independent protection, which was evidenced by more-rapid clearance of the serotype VI strain from the lungs. **Immunization** of mice with tetanus toxoid-type III polysaccharide conjugate did not produce protection, confirming that protection induced by SCPB conjugates was independent of type III polysaccharide antigen. Histol. evaluation of lungs from infected mice revealed that pathol. in animals **immunized** with SCPB or SCPB conjugates was significantly less than that in animals **immunized** with a tetanus toxoid-polysaccharide conjugate. These expts. suggest that inclusion of C5a peptidase in a **vaccine** will both add another level to and broaden the spectrum of the protection of a polysaccharide **vaccine**.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 4 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2002:415220 HCAPLUS
DOCUMENT NUMBER: 139:162926
TITLE: The group B streptococcal C5a peptidase is both a specific protease and an invasin. [Erratum to document cited in CA137:45085]
AUTHOR(S): Cheng, Qi; **Stafslien, Deborah**; Purushothaman, Sai Sudha; **Cleary, Patrick**
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Infection and Immunity (2002), 70(6), 3309
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The article was originally intended to be published together with that of Christiane Beckmann, Joshua D. Wagonner, Theresa O. Harris,

Glen. S. Tamura, and Craig E. Rubens, "Identification of Novel Adhesins from Group B Streptococci by Use of Phage Display Reveals that C5a Peptidase Mediates Fibronectin Binding", ibid. 70 (5), 2869-2876, 2002.

L8 ANSWER 5 OF 16 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002191252 EMBASE
TITLE: Erratum: The group B streptococcal C5a peptidase is both a specific protease and an invasin (Infection and Immunity (2002) 70:5 (2408-2413)).
AUTHOR: Cheng Q.; **Stafslien D.**; Purushothaman S.S.; **Cleary P.**
CORPORATE SOURCE: Q. Cheng, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455, United States
SOURCE: Infection and Immunity, (2002) 70/6 (3309). ISSN: 0019-9567 CODEN: INFIBR
COUNTRY: United States
DOCUMENT TYPE: Journal; Errata
FILE SEGMENT: 004 Microbiology
LANGUAGE: English

L8 ANSWER 6 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 5

ACCESSION NUMBER: 2002:316387 HCAPLUS
DOCUMENT NUMBER: 137:45085
TITLE: The group B streptococcal C5a peptidase is both a specific protease and an invasin
AUTHOR(S): Cheng, Qi; **Stafslien, Deborah**; Purushothaman, Sai Sudha; **Cleary, Patrick**
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Infection and Immunity (2002), 70(5), 2408-2413 CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The group B streptococcus (GBS) is a major cause of pneumonia, sepsis, and meningitis in neonates and a serious cause of mortality or morbidity in immunocompromised adults. Although these streptococci adhere efficiently and invade a variety of tissue-specific epithelial and endothelial cells, adhesins and invasins are still unknown. All serotypes of GBS studied to date express C5a peptidase (SCPB) on their surface. This investigation addresses the possibility that this relatively large surface protein has addnl. activities. Rabbit anti-SCPB serum inhibited invasion of lung epithelial A549 cells by the serotype Ia strain O90R, suggesting that SCPB is an invasin. This was confirmed by inserting an in-frame 25-amino-acid deletion into the scpB gene. Invasion of HEp2 and A549 human cell lines was significantly reduced by the mutation. Enzyme-linked immunosorbent assays were used to demonstrate that purified SCPB protein binds directly to HEp2 and A549 cells and also binds the extracellular matrix protein fibronectin. Binding was dose dependent and saturable. These results suggested that SCPB is one of several potential invasins essential for GBS colonization of damaged epithelium.

Searcher : Shears 571-272-2528

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 7 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:465514 BIOSIS
DOCUMENT NUMBER: PREV200200465514
TITLE: The immune response to group A **streptococcal** (**GAS**) C5a **peptidase** (**SCPA**) in children.
AUTHOR(S): Shet, Anita [Reprint author]; Kaplan, Edward L. [Reprint author]; Johnson, Dwight R. [Reprint author]; **Cleary, Patrick P.** [Reprint author]
CORPORATE SOURCE: Pediatrics, University of Minnesota, Minneapolis, MN, USA
SOURCE: Pediatric Research, (April, 2002) Vol. 51, No. 4 Part 2, pp. 282A. print.
Meeting Info.: Annual Meeting of the Pediatric Societies'. Baltimore, MD, USA. May 04-07, 2002.
CODEN: PEREBL. ISSN: 0031-3998.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Sep 2002
Last Updated on STN: 4 Sep 2002

L8 ANSWER 8 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:459106 BIOSIS
DOCUMENT NUMBER: PREV200100459106
TITLE: **Streptococcal** C5a **peptidase** **vaccine.**
AUTHOR(S): **Cleary, Paul Patrick** [Inventor, Reprint author]
CORPORATE SOURCE: Shoreview, MN, USA
ASSIGNEE: Regents of the University of Minnesota
PATENT INFORMATION: US 6270775 August 07, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 7, 2001) Vol. 1249, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Sep 2001
Last Updated on STN: 22 Feb 2002
AB Novel **vaccines** for use against beta-hemolytic Streptococcus colonization or infection are disclosed. The **vaccines** contain an immunogenic amount of **streptococcal** C5a **peptidase**, or a fragment or mutant thereof. Also disclosed is a method of protecting a susceptible mammal against beta-hemolytic Streptococcus colonization or infection by administering such a **vaccine**.

L8 ANSWER 9 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 6

Searcher : Shears 571-272-2528

ACCESSION NUMBER: 2001:240605 HCAPLUS
DOCUMENT NUMBER: 135:18381
TITLE: Antibody against surface-bound C5a peptidase is opsonic and initiates macrophage killing of group B streptococci
AUTHOR(S): Cheng, Qi; Carlson, Brian; Pillai, Sub; Eby, Ron; Edwards, Lorri; Olmsted, Stephen B.; **Cleary, Patrick**
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Infection and Immunity (2001), 69(4), 2302-2308
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The capsular polysaccharides of group B streptococci (GBS) are a primary focus of **vaccine** development. Immunogenicity and long-lasting protection are best achieved by conjugating polysaccharides to a T-cell-dependent protein antigen. **Streptococcal** C5a **peptidase** (SCPB) is a conserved surface protein that is expressed by all streptococcal serotypes tested to date, and it is a possible carrier protein that could itself induce a protective immune response. Clearance of GBS from lungs, mucosal surfaces, or blood probably depends on the opsonophagocytic response of tissue-specific macrophages and polymorphonuclear leukocytes (PMNs). In this study, we examined the potential of antibody directed against SCPB from a serotype II strain to enhance the capacity of mouse bone marrow macrophages (from primary cultures) and human PMNs in whole blood to kill GBS in vitro. Our expts. demonstrated that Streptococcus serotypes Ia, Ib, II, III, and V, preopsonized with anti-SCPB antibody, were killed more rapidly by cultured macrophages and PMNs in whole blood than were nonopsonized GBS. The increased rate of killing was accompanied by an increased macrophage oxidative burst. Furthermore, opsonization was serotype transparent. **Immunization** with SCPB conjugated to capsular polysaccharide type III produced polysaccharide-specific antibodies. It is interesting that this antiserum promoted serotype-independent killing of streptococci. These data support the use of SCPB in a GBS polysaccharide conjugate **vaccine**. SCPB not only enhanced the immunogenicity of polysaccharide components of the **vaccine**, but it might also induce addnl. serotype-independent protective antibodies.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 10 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 7
ACCESSION NUMBER: 2000:402011 HCAPLUS
DOCUMENT NUMBER: 133:42170
TITLE: **Streptococcal** C5a **peptidase vaccine**
INVENTOR(S): **Cleary, Paul Patrick; Stafslien, Deborah K.**
PATENT ASSIGNEE(S): Regents of the University of Minnesota, USA
SOURCE: PCT Int. Appl., 94 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000034487	A1	20000615	WO 1999-US28826	19991203
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6355255	B1	20020312	US 1998-206898	19981207
BR 9915988	A	20010904	BR 1999-15988	19991203
EP 1137785	A1	20011004	EP 1999-966013	19991203
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002531584	T2	20020924	JP 2000-586920	19991203
US 2002142009	A1	20021003	US 2001-870122	20010530

PRIORITY APPLN. INFO.:
US 1998-206898 A2 19981207
US 1996-589756 A2 19960122
WO 1999-US28826 W 19991203

AB Novel **vaccines** for use against β-hemolytic Streptococcus colonization or infection are disclosed. The **vaccines** contain an immunogenic amount of a variant of **streptococcal** C5a **peptidase** (SCP). Also disclosed is a method of protecting a susceptible mammal against β-hemolytic Streptococcus colonization or infection by administering such a **vaccine.** SCP delays recruitment of phagocytes and clearance of streptococci from subdermal sites of infections, and is required for colonization of the mouse nasopharynx. Enzymically inactive SCP muteins produced by site-directed mutagenesis, and polynucleotides encoding these SCP proteins are further disclosed.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 11 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 8
ACCESSION NUMBER: 2000:348691 HCAPLUS
DOCUMENT NUMBER: 133:85434
TITLE: Characterization of the Streptococcal C5a peptidase using a C5a-green fluorescent protein fusion protein substrate
AUTHOR(S): **Stafslien, D. K.; Cleary, P. P.**
CORPORATE SOURCE: Department of Microbiology, University of Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Journal of Bacteriology (2000), 182(11), 3254-3258

Searcher : Shears 571-272-2528

CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A glutathione-S-transferase (GST)-C5a-green fluorescent protein (GFP) fusion protein was designed for use as a substrate for the streptococcal C5a peptidase (SCPA). The substrate was immobilized on a glutathione-Sepharose affinity matrix and used to measure wild-type SCPA activity in the range of 0.8 to 800 nM. The results of the assay demonstrated that SCPA is highly heat stable and has optimal activity on the synthetic substrate at or above pH 8.0. SCPA activity was unaffected by 0.1 to 10 mM Ca2+, Mg2+, and Mn2+ but was inhibited by the same concns. of Zn2+. The assay shows high sensitivity to ionic strength; NaCl inhibits SCPA cleavage of GST-C5a-GFP in a dose-dependent manner. Based on previously published computer homol. modeling, four substitutions were introduced into the putative active site of SCPA: Asp130-Ala, His193-Ala, Asn295-Ala, and Ser512-Ala. All four mutant proteins had over 1,000-fold less proteolytic activity on C5a in vitro, as determined both by the GFP assay described here and by a polymorphonuclear cell adherence assay. In addition, recombinant SCPA1 and SCPA49, from two distinct lineages of Streptococcus pyogenes (group A streptococci), and recombinant SCPB, from Streptococcus agalactiae (group B streptococci), were compared in the GFP assay. The three enzymes had similar activities, all cleaving approx. 6 mol of C5a mmol of SCP-1 liter-1 min-1.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 12 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:1320 BIOSIS
DOCUMENT NUMBER: PREV200100001320

TITLE: The group B streptococcal C5a peptidase function both as a specific protease and adhesin.

AUTHOR(S): Cheng, Q. [Reprint author]; **Stafslien, D.** [Reprint author]; Olmsted, S.; Carlson, B. [Reprint author]; **Cleary, P. P.** [Reprint author]
CORPORATE SOURCE: Univ. of Minnesota, Minneapolis, MN, USA

SOURCE: Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2000) Vol. 40, pp. 44. print.
Meeting Info.: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. Toronto, Ontario, Canada. September 17-20, 2000. Interscience Conference on Antimicrobial Agents and Chemotherapy; American Society of Microbiology.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Dec 2000
Last Updated on STN: 21 Dec 2000

L8 ANSWER 13 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on

STN
ACCESSION NUMBER: 1999:71532 BIOSIS
DOCUMENT NUMBER: PREV199900071532
TITLE: **Streptococcal** C5a **peptidase vaccine.**
AUTHOR(S): **Cleary, P. P.** [Inventor]
CORPORATE SOURCE: Shoreview, Minn., USA
ASSIGNEE: REGENTS OF THE UNIVERSITY OF MINNESOTA
PATENT INFORMATION: US 5846547 Dec. 8, 1998
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 8, 1998) Vol. 1217, No. 2, pp. 1507. print.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Mar 1999
Last Updated on STN: 1 Mar 1999

L8 ANSWER 14 OF 16 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:415595 BIOSIS
DOCUMENT NUMBER: PREV199800415595
TITLE: Site directed mutagenesis of the streptococcal C5a peptidase.
AUTHOR(S): **Stafslien, Deborah K.; Cleary, P. Patrick**
CORPORATE SOURCE: Univ. Minnesota, Minneapolis, MN, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (1998) Vol. 98, pp. 59. print.
Meeting Info.: 98th General Meeting of the American Society for Microbiology. Atlanta, Georgia, USA. May 17-21, 1998. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Oct 1998
Last Updated on STN: 2 Oct 1998

L8 ANSWER 15 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 1997:499117 HCAPLUS
DOCUMENT NUMBER: 127:160564
TITLE: Complement C5a peptidase **vaccines** against β-hemolytic Streptococcus
INVENTOR(S): **Cleary, Paul P.**
PATENT ASSIGNEE(S): Regents of the University of Minnesota, USA; Cleary, Paul P.
SOURCE: PCT Int. Appl., 76 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9726008	A1	19970724	WO 1997-US1056	19970121
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5846547	A	19981208	US 1996-589756	19960122
CA 2243755	AA	19970724	CA 1997-2243755	19970121
AU 9715828	A1	19970811	AU 1997-15828	19970121
AU 705732	B2	19990527		
EP 877624	A1	19981118	EP 1997-902076	19970121
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2000513709	T2	20001017	JP 1997-526301	19970121
US 6270775	B1	20010807	US 1998-206800	19981207

PRIORITY APPLN. INFO.:
US 1996-589756 A 19960122
WO 1997-US1056 W 19970121

AB **Vaccines**, and **vaccination** methods, are disclosed for use against β-hemolytic Streptococcus colonization or infection in susceptible mammals. The **vaccines** contain an immunogenic amount of **streptococcal** C5a **peptidase**, or a fragment or mutant thereof. Also disclosed is a method of protecting a susceptible mammal against β-hemolytic Streptococcus colonization or infection by administering such a **vaccine**.

L8 ANSWER 16 OF 16 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 10

ACCESSION NUMBER: 1997:351268 HCAPLUS
DOCUMENT NUMBER: 127:79905
TITLE: Intranasal **immunization** with C5a peptidase prevents nasopharyngeal colonization of mice by the group A Streptococcus
AUTHOR(S): Ji, Yinduo; Carlson, Brian; Kondagunta, Aparna; **Cleary, P. Patrick**
CORPORATE SOURCE: Department Microbiology, University Minnesota, Minneapolis, MN, 55455, USA
SOURCE: Infection and Immunity (1997), 65(6), 2080-2087
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Early inflammatory events are initiated by phased production of C5a and interleukin-8 in tissue. Most serotypes of group A **streptococci** express a surface-bound peptidase (**SCPA**) which specifically cleaves mouse and human C5a chemotaxins. This study investigates the impact of SCPA on colonization of the nasopharyngeal mucosa of mice and evaluates its potential to induce protective immunity. Two strains, serotypes M6 and M49, which contain insertion and deletion mutations in the **SCPA** gene (**scpA**) and represent the two major subdivisions of group A **streptococci**, were characterized and compared in a mouse

intranasal infection model. In this model, SCPA mutants were more rapidly cleared from the nasopharynges of inoculated mice compared with wild-type strains. A 2908-bp fragment of scpA49 gene, obtained by PCR, was ligated to the expression vector pGEX-4T-1 and expressed in Escherichia coli. The affinity-purified ΔSCPA49 protein was highly immunogenic in mice and rabbits. Although the purified ΔSCPA49 immunogen lacked enzymic activity, it induced high titers of rabbit antibodies which were able to neutralize peptidase activity associated with M1, M6, M12, and M49 streptococci in vitro. This result confirmed that antipeptidase antibodies lack serotype specificity. Intranasal **immunization** of mice with the deleted form of the SCPA49 protein stimulated salivary secretory IgA and serum IgG antibodies and reduced the potential of wild-type M1, M2, M6, M11, and M49 streptococci to colonize. These expts. suggest a new approach to **vaccine** development for prevention of streptococcal pharyngitis.

FILE 'HOME' ENTERED AT 12:39:47 ON 20 FEB 2004

Searcher : Shears 571-272-2528

(FILE 'CONFSCI, SCISEARCH' ENTERED AT 15:19:48 ON 20 FEB 2004)

L28 546 SEA ABB=ON PLU=ON "CLEARY P"?/AU
L29 3 SEA ABB=ON PLU=ON "STAFSLIEN D"?/AU
L30 3 SEA ABB=ON PLU=ON L28 AND L29
L31 29 SEA ABB=ON PLU=ON (L28 OR L29) AND ((STREPTOCOCC? OR GAS)(3A) PEPTIDASE OR SCPA(S) STREPTOCOCC?)
L32 5 SEA ABB=ON PLU=ON L31 AND (IMMUNIS? OR IMMUNIZ? OR VACCIN?)
L33 8 SEA ABB=ON PLU=ON L30 OR L32
L34 8 DUP REM L33 (0 DUPLICATES REMOVED)

L34 ANSWER 1 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2003:805161 SCISEARCH
THE GENUINE ARTICLE: 719QN
TITLE: Immune response to group A **streptococcal** C5a **peptidase** in children: Implications for **vaccine** development
AUTHOR: Shet A (Reprint); Kaplan E L; Johnson D R; **Cleary P P**
CORPORATE SOURCE: Univ Minnesota, Sch Med, Dept Pediat, World Hlth Org Collaborating Ctr Reference & Res, 420 Delaware St SE, Minneapolis, MN 55455 USA (Reprint); Univ Minnesota, Sch Med, Dept Pediat, World Hlth Org Collaborating Ctr Reference & Res, Minneapolis, MN 55455 USA; Univ Minnesota, Sch Med, Dept Microbiol, Minneapolis, MN 55455 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF INFECTIOUS DISEASES, (15 SEP 2003) Vol. 188, No. 6, pp. 809-817.
Publisher: UNIV CHICAGO PRESS, 1427 E 60TH ST, CHICAGO, IL 60637-2954 USA.
ISSN: 0022-1899.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 35
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The group A **streptococcal** C5a **peptidase** (**SCPA**) is a major surface virulence protein that facilitates the establishment of local infection by group A **streptococci** (GAS). We measured the human immune response to **SCPA**, using a standardized indirect enzyme-linked immunosorbent assay. Paired acute and convalescent serum samples from children with GAS-associated pharyngitis were assayed, and a strong immune response to **SCPA** was demonstrated that was independent of the infecting M type and the age of the patient. Western blot analysis of bacterial extracts revealed that all tested M types expressed **SCPA**. The immune response to **SCPA** correlated with the anti-streptolysin O and anti-DNase B responses. These data confirm the immunogenicity of **SCPA** in humans. Previous knowledge of SPCA's role in virulence, its highly conserved nature, and the results of mouse protection studies make **SCPA** an ideal **vaccine** candidate for the prevention of GAS disease.

L34 ANSWER 2 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:874772 SCISEARCH

THE GENUINE ARTICLE: 605JQ
TITLE: **Immunization** with C5a peptidase or peptidase-type III polysaccharide conjugate **vaccines** enhances clearance of group B streptococci from lungs of infected mice
AUTHOR: Cheng Q; Debol S; Lam H; Eby R; Edwards L; Matsuka Y; Olmsted S B; **Cleary P P (Reprint)**
CORPORATE SOURCE: Univ Minnesota, Dept Microbiol, MMC 196, 420 Delaware St SE, Minneapolis, MN 55455 USA (Reprint); Univ Minnesota, Dept Microbiol, Minneapolis, MN 55455 USA; Wyeth Lederle Vaccines, Rochester, NY 14586 USA
COUNTRY OF AUTHOR: USA
SOURCE: INFECTION AND IMMUNITY, (NOV 2002) Vol. 70, No. 11, pp. 6409-6415.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0019-9567.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 38

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Group B streptococci (GBS) are among the most common causes of life-threatening neonatal infections. **Vaccine** development since the late 1970s has focused on the capsular polysaccharides, but a safe, effective product is still not available. Our quest for a **vaccine** turned to the **streptococcal** C5a **peptidase** (SCPB). This surface protein is antigenically conserved across most if not all serotypes. A murine model was used to assess the impact of SCPB on clearance of GBS from the lungs of intranasally infected animals. Mutational inactivation of SCPB resulted in more-rapid clearance of streptococci from the lung. **Immunization** with recombinant SCPB alone or SCPB conjugated to type III capsular polysaccharide produced serotype-independent protection, which was evidenced by more-rapid clearance of the serotype VI strain from the lungs. **Immunization** of mice with tetanus toxoid-type III polysaccharide conjugate did not produce protection, confirming that protection induced by SCPB conjugates was independent of type III polysaccharide antigen. Histological evaluation of lungs from infected mice revealed that pathology in animals **immunized** with SCPB or SCPB conjugates was significantly less than that in animals **immunized** with a tetanus toxoid-polysaccharide conjugate. These experiments suggest that inclusion of C5a peptidase in a **vaccine** will both add another level to and broaden the spectrum of the protection of a polysaccharide **vaccine**.

L34 ANSWER 3 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:457884 SCISEARCH
THE GENUINE ARTICLE: 554XA
TITLE: The group B streptococcal C5a peptidase is both a specific protease and an invasin (vol 70, pg 2408, 2002)
AUTHOR: Cheng Q (Reprint); **Stafslien D**; Purushothaman S S; **Cleary P**
CORPORATE SOURCE: Univ Minnesota, Dept Microbiol, Minneapolis, MN

55455 USA (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: INFECTION AND IMMUNITY, (JUN 2002) Vol. 70, No. 6, pp. 3309-3309.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0019-9567.
DOCUMENT TYPE: Errata; Journal
LANGUAGE: English
REFERENCE COUNT: 1

L34 ANSWER 4 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:359077 SCISEARCH
THE GENUINE ARTICLE: 543NH
TITLE: The group B streptococcal C5a peptidase is both a specific protease and an invasin
AUTHOR: Cheng Q; **Stafslien D**; Purushothaman S S; **Cleary P (Reprint)**
CORPORATE SOURCE: Univ Minnesota, Dept Microbiol, MMC 196, Minneapolis, MN 55455 USA (Reprint); Univ Minnesota, Dept Microbiol, Minneapolis, MN 55455 USA
COUNTRY OF AUTHOR: USA
SOURCE: INFECTION AND IMMUNITY, (MAY 2002) Vol. 70, No. 5, pp. 2408-2413.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0019-9567.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 31
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The group B streptococcus (GBS) is a major cause of pneumonia, sepsis, and meningitis in neonates and a serious cause of mortality or morbidity in immunocompromised adults. Although these streptococci adhere efficiently and invade a variety of tissue-specific epithelial and endothelial cells, adhesins and invasins are still unknown. All serotypes of GBS studied to date express C5a peptidase (SCPB) on their surface. This investigation addresses the possibility that this relatively large surface protein has additional activities. Rabbit anti-SCPB serum inhibited invasion of lung epithelial A549 cells by the serotype Ia strain O90R, suggesting that SCPB is an invasin. This was confirmed by inserting an in-frame 25-amino-acid deletion into the scpB gene. Invasion of HEp2 and A549 human cell lines was significantly reduced by the mutation. Enzyme-linked immunosorbent assays were used to demonstrate that purified SCPB protein binds directly to HEp2 and A549 cells and also binds the extracellular matrix protein fibronectin. Binding was dose dependent and saturable. These results suggested that SCPB is one of several potential invasins essential for GBS colonization of damaged epithelium.

L34 ANSWER 5 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2001:281023 SCISEARCH
THE GENUINE ARTICLE: 413MT
TITLE: Antibody against surface-bound C5a peptidase is opsonic and initiates macrophage killing of group B

streptococci

AUTHOR: Cheng Q; Carlson B; Pillai S; Eby R; Edwards L; Olmsted S B; **Cleary P (Reprint)**

CORPORATE SOURCE: Univ Minnesota, Dept Microbiol, Box 196 UMHC, Minneapolis, MN 55455 USA (Reprint); Univ Minnesota, Dept Microbiol, Minneapolis, MN 55455 USA; Wyeth Lederle Vaccine, Rochester, NY USA

COUNTRY OF AUTHOR: USA

SOURCE: INFECTION AND IMMUNITY, (APR 2001) Vol. 69, No. 4, pp. 2302-2308.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0019-9567.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The capsular polysaccharides of group B streptococci (GBS) are a primary focus of **vaccine** development, Immunogenicity and long-lasting protection are best achieved by conjugating polysaccharides to a T-cell-dependent protein antigen. **Streptococcal** C5a **peptidase** (SCPB) is a conserved surface protein that is expressed by all streptococcal serotypes tested to date, and it is a possible carrier protein that could itself induce a protective immune response, Clearance of GBS from lungs, mucosal surfaces, or blood probably depends on the opsonophagocytic response of tissue-specific macrophages and polymorphonuclear leukocytes (PMNs), In this study, we examined the potential of antibody directed against SCPB from a serotype II. strain to enhance the capacity of mouse bone marrow macrophages (from primary cultures) and human PMNs in whole blood to kill GBS in vitro, Our experiments demonstrated that Streptococcus serotypes Ia, Ib, II, III, and V, preopsonized with anti-SCPB antibody, were killed more rapidly by cultured macrophages and PMNs in whole blood than were nonopsonized GBS. The increased rate of killing was accompanied by an increased macro phage oxidative burst. Furthermore, opsonization was serotype transparent. **Immunization** with SCPB conjugated to capsular polysaccharide type III produced polysaccharide-specific antibodies. It is interesting that this antiserum promoted serotype-independent killing of streptococci. These data support the use of SCPB in a GBS polysaccharide conjugate **vaccine**. SCPB not only enhanced the immunogenicity of polysaccharide components of the **vaccine**, but it might also induce additional serotype-independent protective antibodies.

L34 ANSWER 6 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

ACCESSION NUMBER: 2000:373384 SCISEARCH

THE GENUINE ARTICLE: 313EU

TITLE: Characterization of the streptococcal C5a peptidase using a C5a-green fluorescent protein fusion protein substrate

AUTHOR: **Stafslien D K; Cleary P P (Reprint)**

CORPORATE SOURCE: UNIV MINNESOTA, DEPT MICROBIOL, BOX 196 FUMC, 420 DELAWARE ST SE, MINNEAPOLIS, MN 55455 (Reprint);

UNIV MINNESOTA, DEPT MICROBIOL, MINNEAPOLIS, MN 55455
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BACTERIOLOGY, (JUN 2000) Vol. 182, No. 11, pp. 3254-3258.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 31

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A glutathione-S-transferase (GST)-C5a-green fluorescent protein (GFP) fusion protein was designed for use as a substrate for the streptococcal C5a peptidase (SCPA), The substrate was immobilized on a glutathione-Sepharose affinity matrix and used to measure wild-type SCPA activity in the range of 0.8 to 800 nM, The results of the assay demonstrated that SCPA is highly heat stable and has optimal activity on the synthetic substrate at or above pH 8.0. SCPA activity was unaffected by 0.1 to 10 mM Ca2+, Mg2+, and Mn2+ but was inhibited by the same concentrations of Zn2+, The assay shows high sensitivity to ionic strength; NaCl inhibits SCPA cleavage of GST-C5a-GFP in a dose-dependent manner. Based on previously published computer homology modeling, four substitutions were introduced into the putative active site of SCPA: Asp(130)-Ala, His(193)-Ala, Asn(295)-Ala, and Ser(512)-Ala. All four mutant proteins had over 1,000-fold less proteolytic activity on C5a in vitro, as determined both by the GFP assay described here and by a polymorphonuclear cell adherence assay. In addition, recombinant SCPA1 and SCPA49, from two distinct lineages of Streptococcus pyogenes (group A streptococci), and recombinant SCPB, from Streptococcus agalactiae (group B streptococci), were compared in the GFP assay. The three enzymes had similar activities, all cleaving approximately 6 mol of C5a mmol of SCP-1 liter(-1) min(-1).

L34 ANSWER 7 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1998:832107 SCISEARCH
THE GENUINE ARTICLE: 132HT
TITLE: Impact of M49, mrp, enn, and C5a peptidase proteins on colonization of the mouse oral mucose by Streptococcus pyogenes
AUTHOR: Ji Y D; Schnitzler N; DeMaster E; **Cleary P (Reprint)**
CORPORATE SOURCE: UNIV MINNESOTA, DEPT MICROBIOL, BOX 196 FUMC, MINNEAPOLIS, MN 55455 (Reprint); UNIV MINNESOTA, DEPT MICROBIOL, MINNEAPOLIS, MN 55455; UNIV HOSP AACHEN, NATL REFERENCE LAB STREPTOCOCCI, AACHEN, GERMANY; UNIV HOSP AACHEN, INST MED MICROBIOL, AACHEN, GERMANY
COUNTRY OF AUTHOR: USA; GERMANY
SOURCE: INFECTION AND IMMUNITY, (NOV 1998) Vol. 66, No. 11, pp. 5399-5405.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0019-9567.

Searcher : Shears 571-272-2528

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 31
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Resistance to phagocytosis is a hallmark of virulent Streptococcus pyogenes (group A **streptococcus**), Surface bound C5a **peptidase** reduces recruitment of phagocytes to the site of infection, and hyaluronic acid capsules and/or the M protein limit the uptake of streptococci, In this study the relative impact of M and M-like proteins and the C5a peptidase on the virulence of a serotype M49 strain was assessed. The capacities of isogenic strains with an insertion mutation in emm49; with a deletion mutation in scpA49 (C5a peptidase gene); and,with a deletion that removes all three M-like genes, mrp49, emm49, and enn49, to colonize mice and resist phagocytosis were compared. Experiments confirmed results obtained in an earlier study, which showed that the M49 protein was not required for in vitro resistance to phagocytosis, and also showed that the M protein was not required for colonization of mice. Failure to produce all three M-like proteins, M49, Mrp, and Enn49, significantly reduced the ability of these streptococci to resist phagocytosis in vitro but did not significantly alter the persistence of streptococci on the oral mucosa, In vitro experiments indicate that M+ streptococci are phagocytized by polymorphonuclear leukocytes that have been activated with phorbol-12-myristate 13-acetate or recombinant human C5a, This observation may explain the finding that expression of M49 protein is not essential for short-term colonization of the mouse oral mucosa.

L34 ANSWER 8 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 97:428679 SCISEARCH
THE GENUINE ARTICLE: XB562
TITLE: Intranasal **immunization** with C5a peptidase prevents nasopharyngeal colonization of mice by the group A Streptococcus
AUTHOR: Ji Y D; Carlson B; Kondagunta A; **Cleary P P (Reprint)**
CORPORATE SOURCE: UNIV MINNESOTA, DEPT MICROBIOL, BOX 196 UMHC, MINNEAPOLIS, MN 55455 (Reprint); UNIV MINNESOTA, DEPT MICROBIOL, MINNEAPOLIS, MN 55455
COUNTRY OF AUTHOR: USA
SOURCE: INFECTION AND IMMUNITY, (JUN 1997) Vol. 65, No. 6, pp. 2080-2087.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0019-9567.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 26
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Early inflammatory events are initiated by phased production of C5a and interleukin-8 in tissue. Most serotypes of group A **streptococci** express a surface-bound peptidase (**SCPA**) which specifically cleaves mouse and human C5a chemotaxins. This

study investigates the impact of **SCPA** on colonization of the nasopharyngeal mucosa of mice and evaluates its potential to induce protective immunity. Two strains, serotypes M6 and M49, which contain insertion and deletion mutations in the **SCPA** gene (**scpA**) and represent the two major subdivisions of group A **streptococci**, were characterized and compared in a mouse intranasal infection model. In this model, **SCPA** mutants were more rapidly cleared from the nasopharynges of inoculated mice compared with wild-type strains. A 2,908-bp fragment of scpA49 gene, obtained by PCR, was ligated to the expression vector pGEX-4T-1 and expressed in Escherichia coli. The affinity-purified Delta SCPA49 protein proved to be highly immunogenic in mice and rabbits. Although the purified Delta SCPA49 immunogen lacked enzymatic activity, it induced high titers of rabbit antibodies which were able to neutralize peptidase activity associated with M1, M6, M12, and M49 **streptococci** in vitro. This result confirmed that antipeptidase antibodies lack serotype specificity. Intranasal **immunization** of mice with the deleted form of the SCPA49 protein stimulated significant levels of specific salivary secretory immunoglobulin A (IgA) and serum IgG antibodies and reduced the potential of wild-type M1, M2, M6, M11, and M49 **streptococci** to colonize. These experiments suggest a new approach to **vaccine** development for prevention of **streptococcal** pharyngitis.

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